EFFECTS OF DAYLENGTH AND TEMPERATURE ON THE REPRODUCTION OF **Heterandria formosa**

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1969

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the members of my supervisory committee: Drs. E. G. Franz Sauer, Archie F. Carr, John Henry Davis, and Hugh L. Popenoe. I am especially grateful to my chairman, Dr. Sauer, for his advice and guidance in preparing the manuscript.

I would like to thank Paul Maslin, Gina Maslin, Dale Burnside, and Jim Brown for their assistance during the field work. At various times Charles Harper and George Phanuel took care of the experimental animals. I am indebted to Paul Laessle, the staff artist, for his suggestions in preparation of the illustrations. Dr. Frank Nordlie was helpful both with suggestions and in procuring the necessary equipment.

I wish to thank the National Science Foundation for their financial support of this study through a National Science Foundation Graduate Fellowship, grant number 6214A43.

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INTRODUCTION

The objective of my study was to investigate factors controlling seasonal changes in population structures and reproduction in the least killifish, Heterandria formosa, a tropical live-bearer living in the Temperature Zone.

It is advantageous for an organism in a seasonal environment to cease reproductive activity when physical factors hinder successful reproduction. Offspring must be produced when food, temperature, and cover favor their survival. Since physiological preparation for sexual activity requires time, an animal must respond to environmental stimuli that precede the onset of favorable conditions at which offspring are born. Different organisms may choose different sets of stimuli. One may expect that during its evolution a species has selected the most reliable environmental stimulus combination to regulate its reproductive cycle.

In temperate regions the most common stimuli triggering reproductive processes are daylength and temperature. In tropical regions where photoperiod and temperature vary only slightly the controlling factors are often seasonal rainfall and flooding. They are believed to act by modifying the inherent rhythm of the endrocrine glands concerned with reproduction. The hypothalamus, when properly stimulated, produces a hormone that causes the anterior pituitary to release a gonadotrophin which in turn initiates gonadal activity.

Vertebrate reproductive cycles have been reviewed by Bissonnette (1936), Rowan (1938), Bullough (1951, 1957), Donovan and Harris (1955), Hoar (1957), and Harrington (1959b). In surveying the literature, I found that very few groups of teleost fishes had been investigated. Nearly all of the work had been done on spring-spawning of oviparous species in the North Temperate Zone. Often investigators working on the same species reached very different conclusions. Much of this confusion arose from insufficient data or because investigators experimented at different phases of the fishes' annual reproductive cycles. Harrington (1950, 1956, 1957) and Baggerman (1957) found that certain fishes react differently to the same combinations of daylength and temperature at different seasons. In his review of photoperiodism in fishes, Harrington (1959b) pointed out that "meaningful comparisons between experiments can be made only in the context of the annual reproductive cycle as a whole."

Harrington (1947, 1950, 1957) found that _Notropis_ _bifrenatus_ has a post-spawning refractory period during which the fish is physiologically incapable of reproduction. During this time, the eggs grow to a "critical diameter" independently of photoperiod. When they have reached a diameter of 336 microns they will continue to grow only when exposed to a long photoperiod. Although initial development may be completed by late summer or early fall, the eggs cannot mature until the following spring unless they are artifically exposed to long days.

The oogonia of Phoxinus laevis develop into primary oocytes during the summer and fall but do not mature until the following spring (Bullough, 1939, 1940). Vernal maturation is slow in fish under a short photoperiod and rapid when fish are exposed to a long photoperiod. In Phoxinus laevis the internal rhythm appears to be stronger than in Notropis bifrenatus.

Ahsan (1966a, 1966b) working on Couesius plumbeus, found that temperature rather than photoperiod was the dominant environmental factor regulating spermatogenesis. Prior to spawning, low temperature caused the fish to retain sperm, while high temperature caused them to shed sperm, and their testes regressed to a post-spawning condition. In post-spawning fish low temperature promoted rapid proliferation of primary spermatocytes from spermatogonia, while high temperature halted mitotis at the level of secondary spermatogonia. No combinations of light and temperature would induce this species to breed before its normal spawning period. Ahsan interpreted this to mean that a strong endogenous rhythm of reproductive activity affects the lake chub. Though timed by temperature and photoperiod, the rhythm is not completely governed by them.

When kept under a 16-hour day at 20°C both sexes of Gasterosteus aculeatus followed a 200-day reproductive cycle consisting of a long non-reproductive period followed by a short period of reproduction (Baggerman, 1957). As in the cyprinids, high temperature combined with short days inhibited oogenesis.

Earlier workers on Gasterosteus drew different conclusions. Courrier (1922) and Craig-Bennet (1931) believed that temperature was the main regulatory factor and that photoperiod had no effect. Vanden Eeckhoudt (1946) held sticklebacks with mature but quiescent testes at 10°C and found that this state was maintained under long days; spermatogenic activity was resumed when the days were short. In contrast, Kazanskii's (1951, 1952) sticklebacks would not respond at temperatures as low as 10°C. At 20° to 22°C under continuous light or natural winter light they spawned. Although spawning did not occur in constant dark, he concluded that temperature was the dominant factor in controlling reproduction while photoperiod was of minor importance. This confusion will not be resolved until the effects of environmental stimuli on the gonads are studied.

Harrington (1957) found in Enneacanthus obesus, a centrarchid, a sexual cycle similar to that of the cyprinids.

Only one autumn-spawning fish, Salvelinus fontinalis, has been studied. Hoover and Hubbard (1937) induced spawning four months early by first increasing the daylength artificially and then decreasing it, thereby stimulating a contracted year. Hazard and Eddy (1951) induced spawning one month early by decreasing photoperiod only. This indicated that increasing photoperiod might hasten the growth of the earlier stages of gametes thus preparing the way for maturation when daylengths were shortened (Harrington, 1959b). Unlike the spring spawning teleosts, the eastern brook trout had no period of testicular quiescence (Henderson, 1963).

All of the above studies were conducted north of the 41st parallel; only a few tropical and subtropical fishes have been studied.

The darter, Etheostoma lepidum, spawns from November to May on the Edwards Plateau in Texas (Hubbs and Strawn, 1957). In a spring where the water temperature remains below 24°C the darter breeds throughout the year. In a series of laboratory experiments, daylength and time of year seemed to play little role in controlling the reproductive cycle, and fecundity appeared to be controlled by temperature.

Harrington (1959a) working with Fundulus confluentus from Vero Beach, Florida, found that during the non-breeding season from November to January high temperatures induced later phases of eggs to mature but retarded the earlier phases. Low temperatures accelerated the development of earlier stages and suppressed that of later stages. A photoperiod effect was evident only at low temperatures where long days suppressed the development of early stages of eggs. There was no seasonal cycle of spermatogenesis. Harrington's study appears to indicate that high temperatures are responsible for the initiation of reproduction in the spring.

Finally, the reproductive cycles of the poeciliid fishes are of interest for several reasons. The Poeciliidae are a family of tropical live-bearers; some species have expanded their range into the North and South Temperate Zones. Because they evolved in an environment where physical conditions were lastingly benign, they have been able to compress their reproductive cycle so that many broods can be produced in a single year. Their cycles have been reduced to less than a month in many species. Heterandria formosa, as well as several other species, carry several broods simultaneously (Turner, 1937).

Turner (1957) caused Jenynsia lineata, a live-bearer of the family Jenynsidae, to come into a second reproductive period by shifting the fish from latitude 34°S to 42°N.

Medlen (1951) kept Gambusia affinis in a tank at 27°C and increased the photoperiod from 10 to 17 hours. The fish became gravid sooner than those kept at the same temperature under a 10-hour photoperiod. Fish at 9°C under a 17-hour light cycle did not become gravid. He concluded that water temperature plays a major role in regulating reproduction while daylength seems of minor importance.

Dildine (1936) found no difference between the fecundity of Lebistes reticulatus kept in the dark and those illuminated by artificial light. He, too, concluded that temperature was the major regulatory factor.

Turner (1937) kept records of brood intervals and sizes for Gambusia affinis, Lebistes reticulatus, Platypoecilus maculatus, Poecilistes pleurospilus, and Heterandria formosa raised at room temperature and exposed to the natural photoperiod at Evanston, Illinois. Their brood intervals became long and brood sizes small during the winter months.

Brachyrhaphis episcopi reproduced at a constant rate throughout the year in Panama (Turner, 1938). Field studies in the United States showed that Gambusia affinis ceased reproduction during the winter months (Kuntz, 1913; Barney and Anson, 1921; Self, 1940: and Krumholtz, 1948). McLane (1955) surveyed the St. John's River in Florida and found that Heterandria formosa, Gambusia affinis, and Mollienesia latipinna regularly ceased reproduction from November to February. In springs along the river Heterandria and Mollienesia ceased to reproduce during these months though the temperature remained above 69°F. Gambusia, however, remained reproductively active throughout the winter.

Turning to the objective of my study, a few facts about Heterandria formosa should be recalled. The least killifish is distributed from Berkely County, South Carolina, southward along the coastal plain into peninsular Florida and as far west as New Orleans, Louisiana (Rosen and Bailey, 1963). It is the smallest vertebrate in North America. Adult males measure 15.5 to 19.0 mm in total length. Adult females range from 14.0 to 42.5 mm in length, although they seldom exceed 30 mm. In mature males the third, fourth, and fifth anal rays are elongated to form a copulatory organ, the gonopodium (Rosen and Gorden, 1953) Sperm from a single copulation can be stored for 10 or more months within the folds of the oviduct (Turner, 1947). As in all poeciliids, the embryos are retained until parturition within the ovarian follicles (Turner, 1937).

FIELD STUDIES

Introduction

The field studies were conducted to examine seasonal changes in the major factors affecting the reproductive efficiency of the least killifish, growth and maturation rates, longevity, sex ratios, and brood production. The effects of daylength and temperatures on these factors have been investigated.

Materials and Methods

Monthly collections of _Heterandria_ _formosa_ were taken from Lakes McCloud and Anderson Cue between November 1, 1967, and November 1, 1968. The lakes are located in a sandhill community of turkey oak and longleaf pine 4 miles southeast of Melrose, Florida. The lakes are elliptical in shape, approximately 20 acres in area, and 15 to 20 feet deep. They were formed by solution of the underlying limestone and are perched on the Hawthorne formation 20 to 30 feet above the Florida aquifer. Because the soil is sandy and porous there is little surface run-off, the water level being determined by a balance between gain from precipitation and seepage and loss by surface evaporation and outflow into the Florida aquifer. The water levels fluctuate considerably from wet to dry periods. Both lakes are oligotrophic with very soft water and have sandy bottoms.

Lake McCloud has an extensive littoral zone, 20 to 40 feet wide, while that of Anderson Cue is only 10 to 15 feet wide. As the water level falls the width of the littoral decreases. It is characterized by an inner zone of Saint John's Wort (*Hypericum* sp.) and common buttonbush (*Cephalanthus occidentalis*), both common in water up to 1½ feet deep, as well as on the wet shore. *Lythrum* sp., (*Mayaca aubleti*), cut grass (*Leersia hexandria*), a proliferating sedge (*Eleocharis acicularis*), and maiden cane (*Panicum hemitomum*) form dense mats of vegetation around the woody shrubs in water up to 3 feet deep. Water shield (*Brasenia shreberi*), *Sagittaria graminea*, and *Nymphoides aquaticum* are sparsely distributed throughout this zone. Dense beds of cut grass are located between this zone and the open water. The least killifish lives in the zone of *Mayaca* and sedge.

Lakes McCloud and Anderson Cue also contain large-mouth bass (*Micropterus salmoides*), blue gills (*Lepomis macrochirus*), warmouth (*Chaenobryttus gulosus*), brook silver-sides (*Labidethes sicculus*), Florida swamp darters (*Etheostoma baratti*) and mosquito-fish (*Gambusia affinis*). In addition Lake McCloud harbors golden topminnows (*Fundulus chrysotus*), and eastern star-headed topminnows (*Fundulus notti*).

Mosquito-fish prey on young least killifish which stray from the dense mats of *Mayaca* and sedge. Blue gills, warmouth, and young large-mouth bass prey on the adults. Predation is severe during periods of prolonged drought. In the spring of 1968 the water level dropped several feet causing the Anderson Cue littoral to nearly disappear. From then on least killifish were scarce until the spring of 1969.

The least killifish feed almost exclusively on Cladocera, but when these become scarce, rotifers constitute the bulk of their diet. The swamp darter, the golden topminnow, and the mosquito-fish compete with the least killifish for food.

The least killifish were collected by sweeping a fine-meshed dip net through the dense vegetation. The same section of the littoral was sampled each month. Samples varied from 50 to 444 individuals depending on the abundance of the fish. Even the smallest individuals were netted by the fine mesh. The fish were fixed in 10 per cent formalin.

The total length of the fish was measured to the nearest 0.5 mm, and fish over 10.0 mm were sexed. Adult males were recognized by their gonopodium and females by the dark spot on their anal fin. All small fish unidentifiable externally were sexed by internal examination. Criteria were the ovary in females and the conspicuous erector analis major muscle in males. Monthly population structures and sex ratios were recorded.

The age of the females collected during the summer months was determined by counting the annuli on their scales. By this means the production of embryos per overwintering female could be compared with that of females born in the spring.

The ovaries from all adult females were dissected and the embryos were classified according to their developmental state:

1. Embryo 0.75 to 1.3 mm in diameter; transparent and jelly-like in appearance. Eyes absent or if present very faintly pigmented. Body outline indistinct.

2. Embryo 1.4 to 1.7 mm in diameter; lightly colored body. Eyes darkly pigmented; body outline indistinct.
3. Embryo 1.8 to 2.5 mm in diameter; body outline and fins distinct. Bars on sides of body faintly visible. Yolk sac large.
4. Embryo over 2.5 mm in diameter; fully formed and ready for birth. Bars on sides of body dark. Yolk sac small or absent.

The testes were removed from a sample of 10 mature males, fixed in 10 per cent formalin, sectioned, and stained with Heidenhain's iron hematoxylin according to a procedure derived from Pantin (1962). The slides were mounted and photomicrographs were taken with a Leitz-Orthomatic camera. Testes from males collected in different months of the year were compared to study spermatogensis.

In addition to the collections made in Lakes McCloud and Anderson Cue, a single collection was made at Ichatucknee Springs on February 2, 1969. The water of Ichatucknee Springs remains approximately 21°C year around and supports a small population of least killifish. This sample was compared to those taken from the lakes to study the effects of warm water on reproduction during the winter months.

Results

Growth and Maturation

The lengths of the fish from each sample have been plotted, females in Figures 1 and 2 and males in Figures 3 and 4. The first young were born in Lake McCloud shortly after the April 5 collection (Fig. 1). By May 2 the females were 13.0 mm long. Since the least

Figure 1. Total lengths of female least killifish over 10.0 mm long taken in monthly collections from Lake McCloud. N = sample size; stippled areas = gravid females, unstippled areas = non-gravid females.

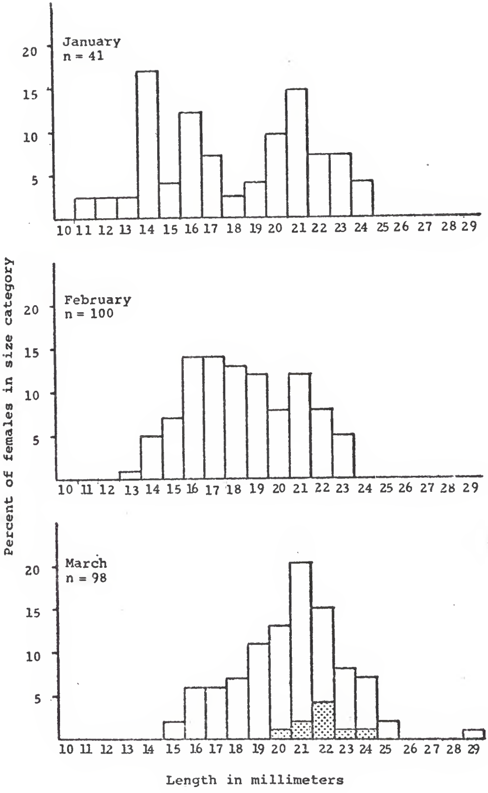

January
n = 41
February
n = 100
March
n = 98
20
15
10
5
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Percent of females in size category
Length in millimeters

Figure 1 (continued)

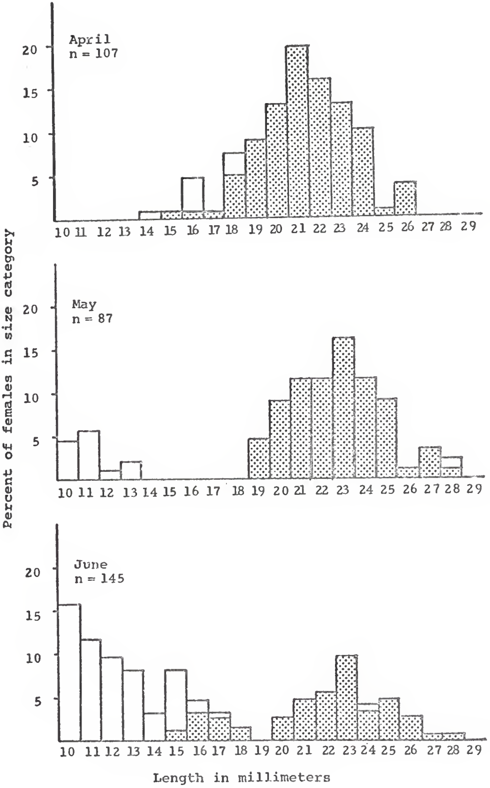


Figure 1 (continued)

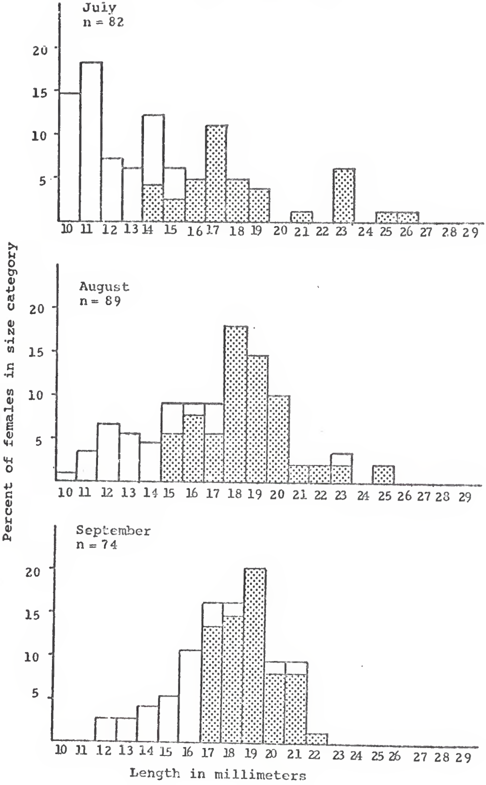


Figure 1 (continued)

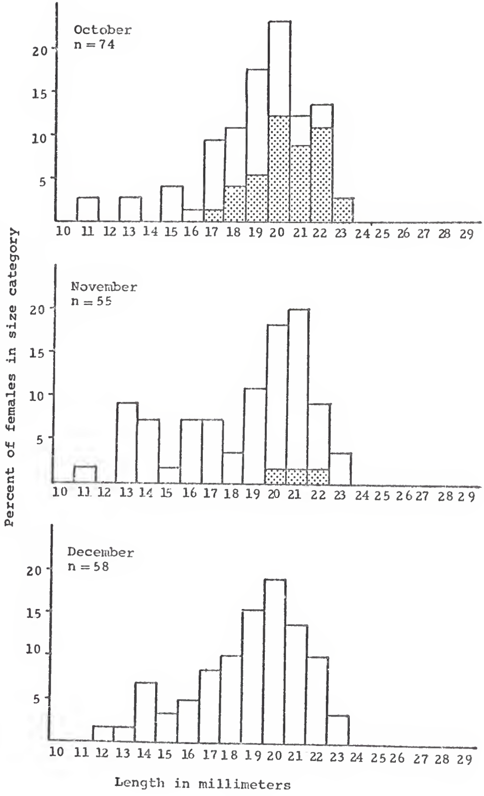


Figure 2. Total lengths of female least killifish over 10.0 mm long taken in monthly collections from Lake Anderson Cue. N = sample size, stippled areas = gravid females, unstippled areas = non-gravid females.

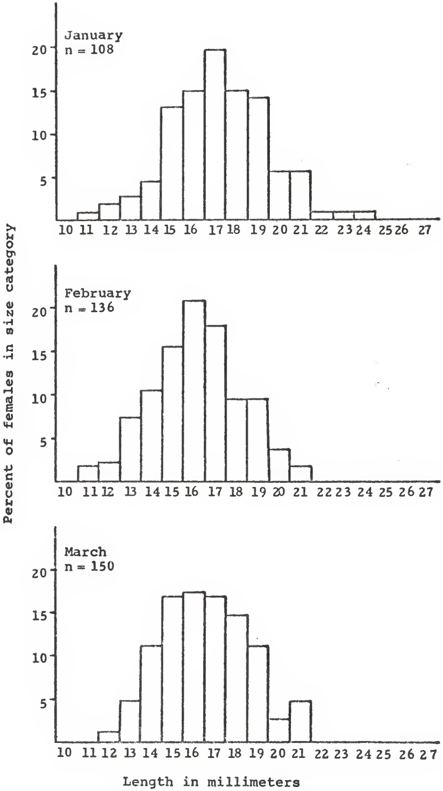
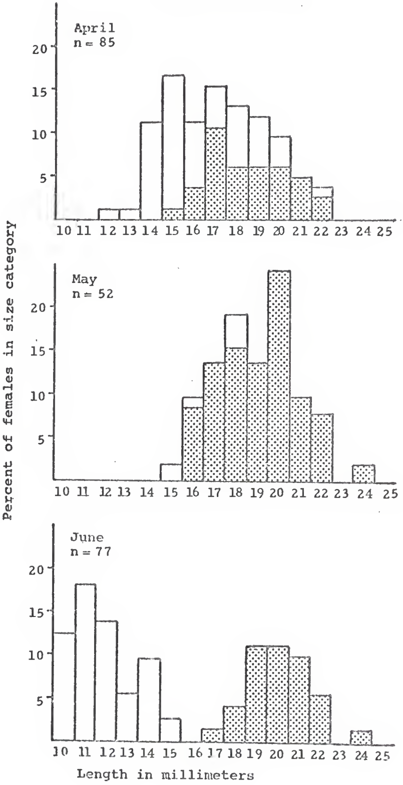

January
n = 108
February
n = 136
March
n = 150
20
15
10
5
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Percent of females in size category
Length in millimeters

Figure 2 (continued)



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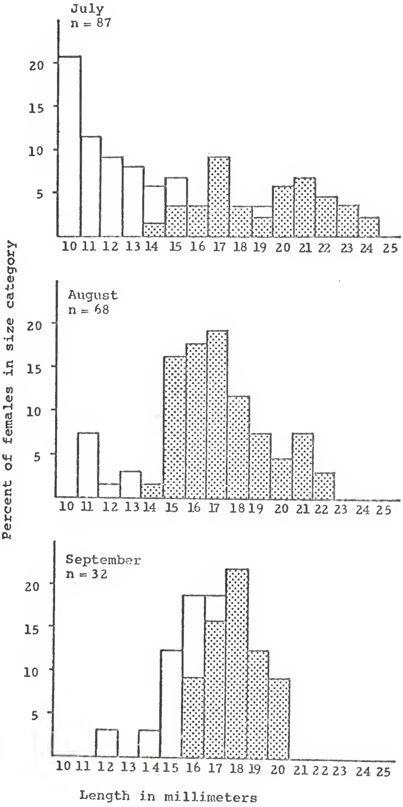


Figure 2 (continued)

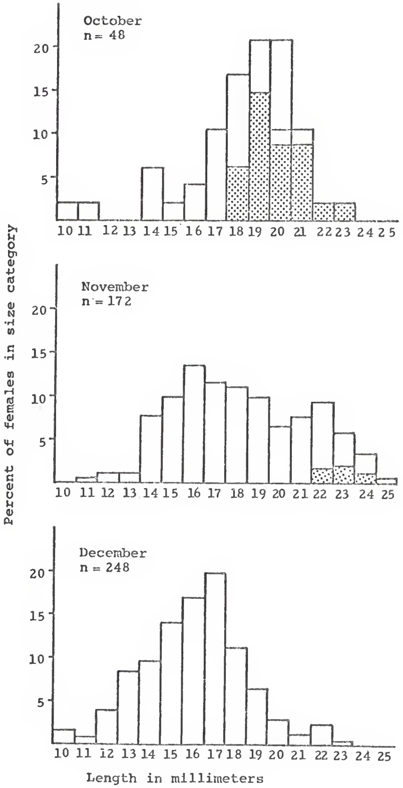


Figure 3. Total lengths of male least killifish over 10.0 mm long taken in monthly collections from Lake McCloud. N = sample size, stippled areas = mature males, unstippled areas = immature males.

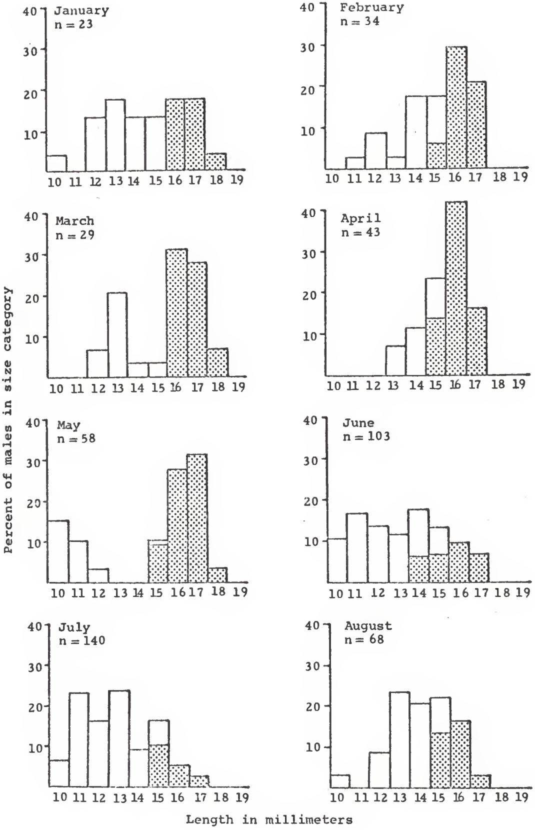

January
n = 23
February
n = 34
March
n = 29
April
n = 43
May
n = 58
June
n = 103
July
n = 140
August
n = 68
40
30
20
10
10 11 12 13 14 15 16 17 18 19
Percent of males in size category
Length in millimeters

Figure 3 (continued)

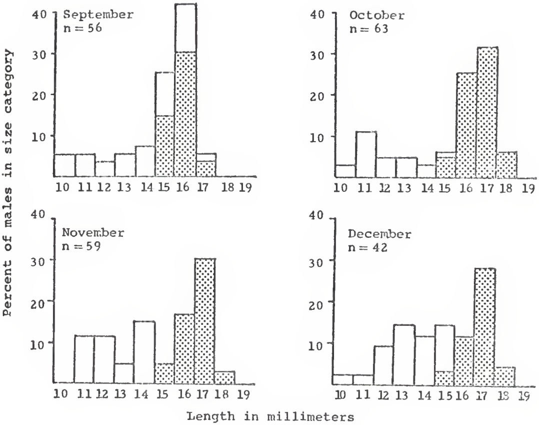


Figure 4. Total lengths of male least killifish over 10.0 mm long taken in monthly collections from Lake Anderson Cue. N = sample size, stippled areas = mature males, unstippled areas = immature males.

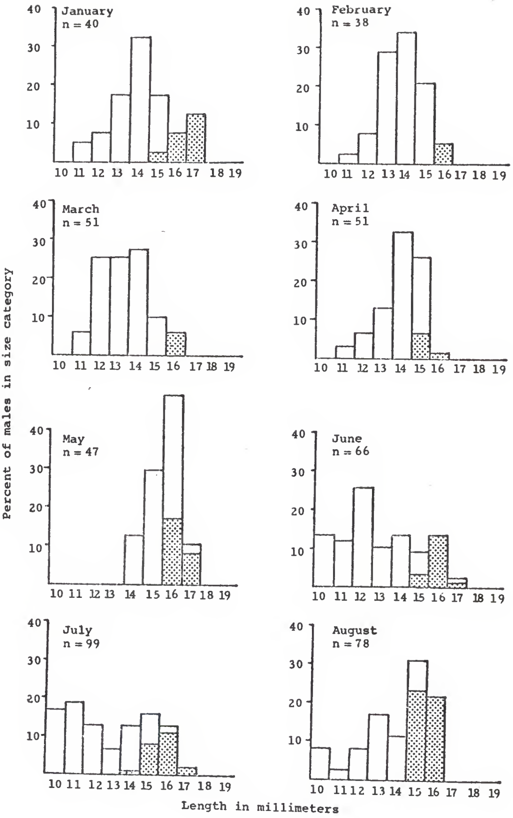
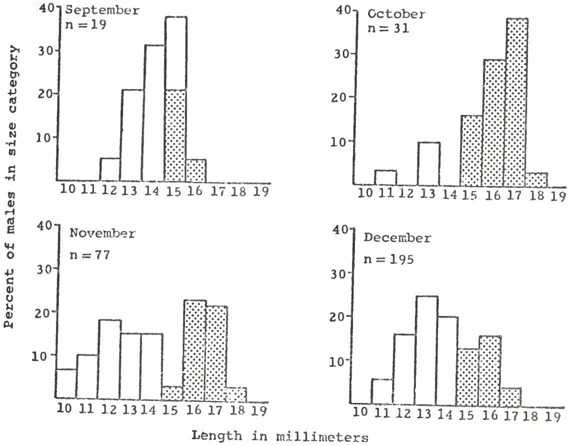

January
n = 40
February
n = 38
March
n = 51
April
n = 51
May
n = 47
June
n = 66
July
n = 99
August
n = 78
10
20
30
40
10 11 12 13 14 15 16 17 18 19
Percent of males in size category
Length in millimeters

Figure 4 (continued)



killifish are about 7.0 mm long at birth, these young females had grown roughtly 6.0 mm during the first month. The growth rates in their second, third, and fourth monthswere 5.0, 3.0, and 2.0 mm, respectively. By May 2 the males were 12.0 mm long, having increased 5.0 mm in length during the first month (Fig. 3). After May 2 the new males could no longer be distinguished from the old males.

The females reached sexual maturity and contained fertilized eggs in about 35 days when they were 14.0 mm long. Males became sexually mature at a length of 15.0 mm, which they had reached about 45 days after birth. Unlike females, males born during the fall did not become sexually mature until the following spring (Figs. 5 and 6).

Females grew slowly during the non-reproductive season from November to April (Figs. 1 and 2). During the month of April the females in both lakes grew rapidly; females 14.0 mm long grew at least 5.0 mm and those over 20.0 mm added at least 2.0 mm. Like the females, males grew little or not at all during the non-reproductive season but showed rapid growth during April (Figs. 3 and 4).

The largest male taken from the lakes was 18.0 mm long. Apparently males stopped growing shortly after they reached sexual maturity. Females, however, never ceased growing. Although the largest female taken from the lakes was only 29.0 mm long, I caught one 42.5 mm long in a small pond near Anderson Cue on April 5, 1968. The presence of only one annulus on the scales suggested that she was less than a year old.

Figure 5. Percentage of immature least killifish over 10.0 mm long taken in monthly collections from Lake McCloud. Unbroken line = males, dotted line = females.

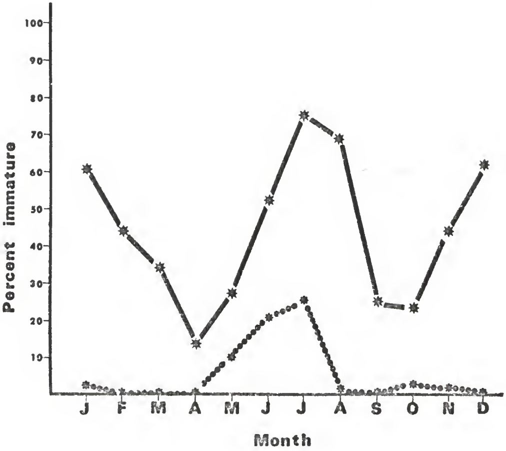
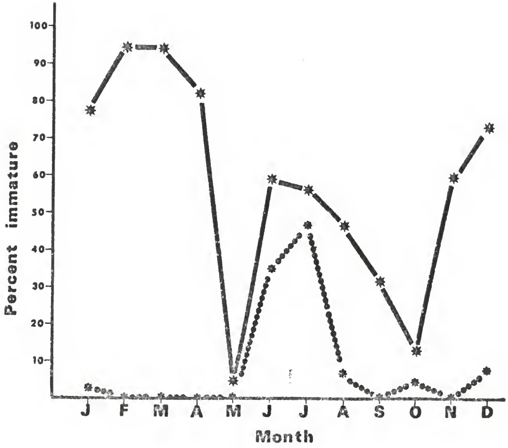

Percent immature
100
90
80
70
60
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D
Month

Figure 6. Percentage of immature least killifish over 10.0 mm long taken in monthly collections from Lake Anderson Cue. Unbroken line = males, dotted line = females.


Percent immature
100
90
80
70
60
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D
Month

Only three non-gravid females more than 20 mm long were taken between May 1 and August 1. Two of these contained single large nematodes which occupied most of the body cavity. As most of the overwintering females died during this period, post-reproductive senility seems unlikely in the least killifish.

Sex Ratios

The sex ratios varied seasonally. The ratio of mature females to mature males was high from December to April and reached a peak during February and March (Table 1). The February 1 collection from Lake Anderson Cue contained 120 mature females and two mature males, a ratio of 60:1. In May, June, and July the sex ratio was balanced in both lakes.

The ratio of immature females to immature males also showed a predominance of females during the winter and early spring and became approximately 1:1 during the summer (Table 2). The ratios during the winter months did not depart so markedly from unity as did the ratios of sexually mature fish. There was a peak of 3.55 females per male during February in Lake Anderson Cue.

In an effort to learn whether the predominance of females could be explained by a differential birth rate, all individuals 10 to 13 mm long were sexed. In the Lake McCloud collections I had 151 males and 121 females, a ratio of 1.25 males per female. From Lake Anderson Cue I obtained 102 males and 79 females, a ratio of 1.29 males per female. It is possible that more males than females were

Table 1

Monthly sex ratios of mature Heterandria formosa.

Month	Lake McCloud			Anderson Cue		
	Males	Females	Ratio x ♀:100 ♂	Males	Females	Ratio x ♀:100 ♂
January	9	38	423	9	102	1133
February	19	99	521	2	120	6000
March	19	98	516	3	143	4770
April	37	107	289	9	83	923
May	42	70	167	45	52	116
June	49	79	161	27	41	152
July	40	44	110	43	47	109
August	21	74	356	42	60	143
September	42	70	167	13	31	238
October	48	70	146	27	46	170
November	33	49	148	31	166	535
December	16	55	344	52	210	404

Table 2

Monthly sex ratios of all Heterandria formosa whose sex could be identified.

	Lake McCloud			Anderson Cue		
Month	Males	Females	Ratio x ♀:100 ♂	Males	Females	Ratio x ♀:100 ♂
January	23	41	178	40	105	262
February	34	100	294	38	135	355
March	29	98	338	51	150	294
April	43	107	249	51	85	167
May	58	87	150	47	52	110
June	103	139	135	66	57	86
July	140	82	59	99	60	67
August	68	89	131	78	64	82
September	56	74	132	19	32	168
October	63	74	117	31	46	148
November	59	55	93	77	169	220
December	42	58	138	195	232	119

born, that females had a higher death rate than males, or that they grew faster than males. The third hypothesis is supported by my data (p. 28).

Since sexing the individuals of two weeks to one month of age evidenced no predominance of females, their excessive numbers during the winter months might be explained by a high death rate for males. Males were indeed more susceptible to adverse temperature conditions than females (p.77).

After the February peak the sex ratio rapidly became balanced. As the ratio of females to males decreased long before young were born, one must infer that during the spring the females had a higher death rate than the males.

Sperm Production

Quantitative differences in the condition of the testes were noticed only during May and June when fewer mature spermatozoa were present. Even during these months sufficient quantities of sperm were present for insemination of females and the males engaged actively in courtship behavior.

Production of Embryos

Females whose eggs were fertilized around March 1 gave birth on April 5. This indicates a gestation period of 35 days. The offspring born on April 5 gave birth 60 days later. As 35 days were required to reach sexual maturity (p. 28), their gestation period must have been about 25 days. The gestation period is indeed shorter at higher temperatures (p. 77).

As many as six broods of one to 11 embryos developed simultaneously in a single female. One laboratory-reared female (25.0 mm long) contained 32 embryos, i.e, from four broods of 11, four, eight, and nine embryos.

The average number of embryos per female was directly proportional to the length of the female (Fig. 7). Reproductive maturity corresponded to a total length of 14.5 mm. On the average the females contained 1.435 embryos for each additional millimeter of length.

The first gravid females were found in Lake McCloud on March 1 and in Lake Anderson Cue on April 5 (Fig. 8). During the first month the average number of embryos per female was low as few females were gravid (Fig. 9), and they contained only embryos in the earliest stages of development (Tables 3 and 4). The peak in the average number of embryos per female during May and June could be attributed to the large size of the gravid females (Figs. 1 and 2) and to the high per cent of females which contain embryos (Fig. 9).

The distributions of the lengths of young females in the June and July collections were cyclical (Figs. 1 and 2). This could be the result of a cyclical pattern of births (p. 64).

The gradual replacement of the large, old females by the smaller, new females (Fig. 10) can explain most of the decrease in the average

Figure 7. The average number of embryos per female in relation to the total length of the female. The fish were collected during May, June, and July, 1968, in Lake McCloud.

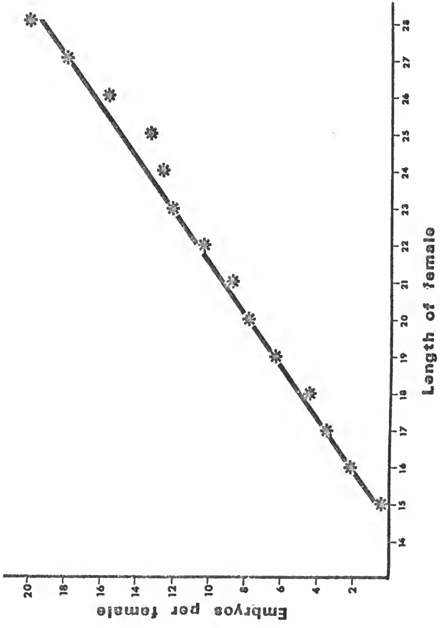

Embryos per female
2
4
6
8
10
12
14
16
18
20
Length of female
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Figure 8. The average number of embryos per female for fish over 14.0 mm. Dotted line = production by Lake McCloud females, unbroken line = production by Lake Anderson Cue females.

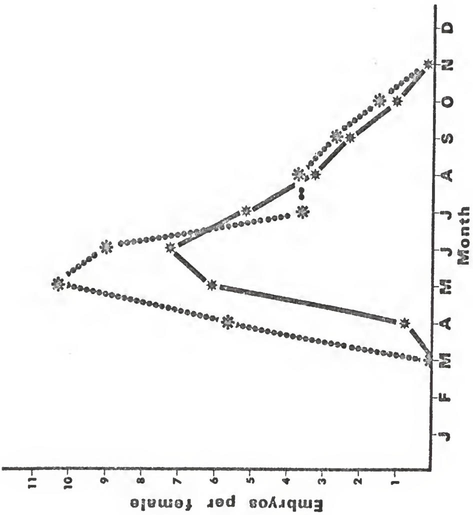

Embryos per female
1
2
3
4
5
6
7
8
9
10
11
J
F
M
A
M
J
J
A
S
O
N
D
Month

Figure 9. Percentage of females over 14.0 mm long containing embryos. Dotted line = production by Lake McCloud females, unbroken line = production by Lake Anderson Cue females.

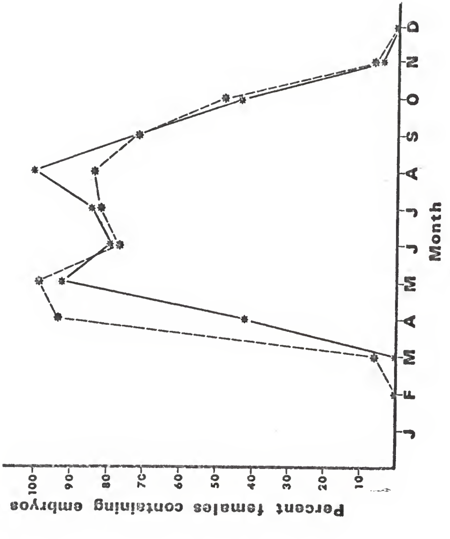

Percent females containing embryos
100
90
80
70
60
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D
Month

Figure 10. Percentage of females in Lake McCloud which have overwintered.

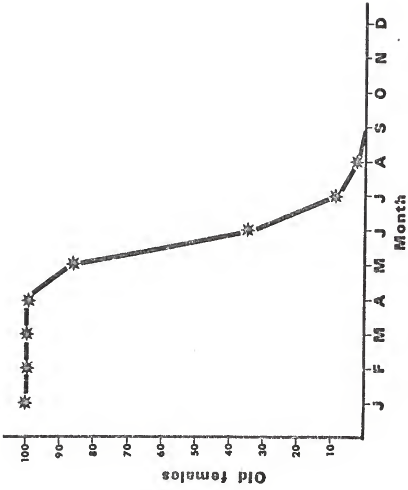

Old females
100
90
80
70
60
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D
Month

Table 3

Seasonal variation in percentage of embryos in various stages of development from females taken in Lake McCloud.

Month	Per cent embryos in age category 1	2	3	4	Average number of embryos per female
January	-	-	-	-	0.00
February	-	-	-	-	0.00
March	100.0	0.0	0.0	0.0	0.15
April	40.5	36.3	22.7	0.5	5.65
May	23.3	26.3	23.3	27.1	10.30
June	25.3	24.5	26.4	23.8	9.02
July	31.5	24.5	25.1	18.9	3.68
August	27.3	21.3	28.2	24.2	3.53
September	20.7	22.9	24.5	31.9	2.65
October	4.3	6.4	25.8	63.5	1.33
November	0.0	0.0	50.0	50.0	0.08
December	-	-	-	-	0.00

Table 4

Seasonal variation in percentage of embryos in various stages of development from females taken in Lake Anderson Cue.

Month	Per cent embryos in age category 1	2	3	4	Average number of embryos per female
January	-	-	-	-	0.00
February	-	-	-	-	0.00
March	-	-	-	-	0.00
April	55.6	28.1	17.3	0.0	0.75
May	22.5	26.1	25.7	25.7	6.16
June	25.7	22.5	27.1	24.7	5.19
July	24.6	23.1	22.8	28.5	4.03
August	18.2	33.9	34.3	13.6	3.16
September	19.5	26.4	31.9	22.2	2.25
October	6.3	12.5	10.4	70.8	1.04
November	0.0	3.3	16.7	80.0	0.18
December	-	-	-	-	0.00

number of embryos per female from June to September (Fig. 11). As many as 24 embryos were found in old females, but not more than 10 in newly matured females. In Lake McCloud the old females produced 79 per cent of the season's total offspring.

Not all of the difference in fecundity between the old and new females could be explained on the basis of their size. When I compared old females from the May and June collections with new females from August and September when they are equal in size, I found that the average number of embryos per old female was much higher (Table 5). Likewise, plots of number of embryos per gravid female versus total length showed that females produced fewer embryos in August than in June (Figs. 12 and 13).

The first evidence of the end of the reproductive season in Lake McCloud came in the August collection (Fig. 1). The females of 14 to 17 mm showed a marked decline in fertility. This decline was not apparent in the collections from Lake Anderson Cue until September (Fig. 2).

By October eggs of the older females remained unfertilized; embryos in the early stages of development became scarce (Tables 3 and 4). The last offspring of the year were born by mid-November (Fig. 9).

All of the females in the single collection from Ichatucknee Springs (February 2) were gravid and contained on the average 4.78 embryos. Only two immatures were taken, both under 10 mm in total

Figure 11. The number of embryos per female for fish over 14.0 mm long taken in monthly collections from Lake McCloud. Unshaded areas = females which have overwintered, shaded areas = females of the season.

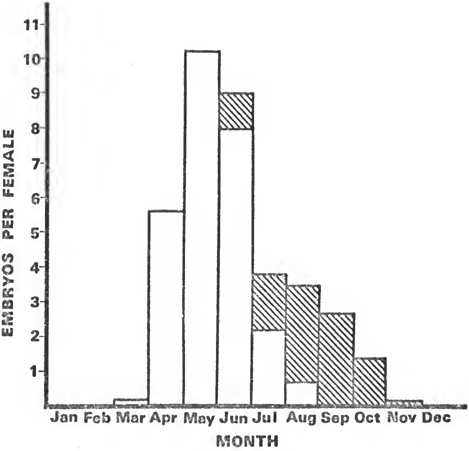

11
10
9
8
7
6
5
4
3
2
1
EMBRYOS PER FEMALE
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
MONTH

Table 5

The average number of embryos per gravid female from Lake McCloud. The numbers to the left of the line indicate embryos produced by females which have overwintered; those to the right refer to the embryos produced by their offspring.

Length	March	April	May	June	July	August	September	October	November
13									
14					1.00				
15		1.00		1.00	1.00	1.00			
16		1.50		1.80	2.50	1.72			
17		3.00		3.00	3.33	3.00	2.10	3.00	
18		4.17		5.00	4.00	4.47	3.30	1.33	
19		2.87	5.50		5.00	4.77	3.53	2.25	
20	1.00	4.00	6.50	9.33		4.22	6.18	2.45	1.00
21	2.00	4.86	8.10	10.40	4.00	6.50	5.84	3.50	1.00
22	2.33	6.36	9.90	14.25		7.50	6.00	3.63	2.00
23	5.00	9.50	11.65	13.50	12.60	7.50		2.50	
24		9.10	12.50	16.00					
25		1.00	11.35	14.85	15.00		9.50		
26		12.50	13.00	17.33	13.00				
27			16.00	24.00					
28			21.00	19.00					

Figure 12. The average number of embryos per gravid female sampled from Lake McCloud. Dotted line = August sample, undotted line = June sample.

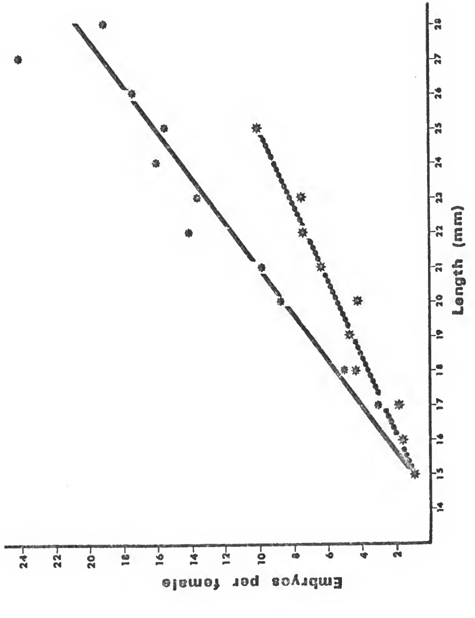
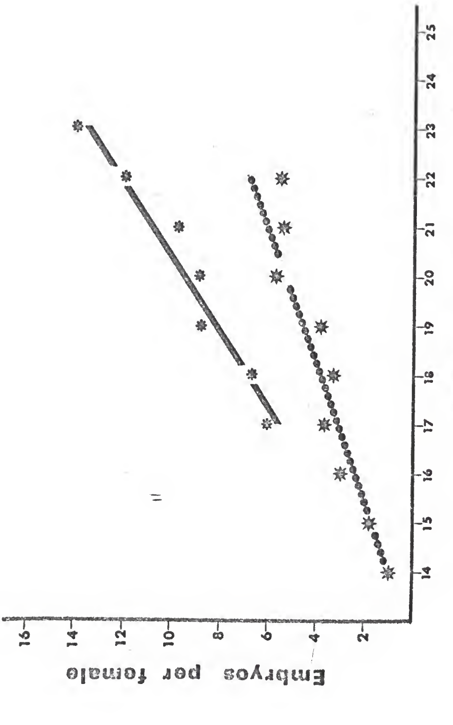

Embryos per female
2
4
6
8
10
12
14
16
18
20
22
24
Length (mm)
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Figure 13. The average number of embryos per gravid female sampled from Lake Anderson Cue. Dotted line = August sample, undotted line = June sample.


II
Embryos per female
16
14
12
10
8
6
4
2
Length (mm)
14
15
16
17
18
19
20
21
22
23
24
25

length. None of the females was from the 10 to 18 mm range. This indicates that the population in the 21°C spring had ceased reproduction for at least two months.

Seasonal Environmental Changes

A graph of the seasonal changes in surface temperature for Anderson Cue is presented in Figure 14. Bottom temperature at a depth of 15 feet was 1°C less than the surface temperature. The times of greatest change in temperature came from February to May and from September to November.

The periods of greatest change in photoperiod came during March and September (Fig. 15) The photoperiod changed during the summer months while temperature remained relatively constant.

The most noticeable change in the availability of food took place during July when the Cladocera disappeared and the least killifish were forced to eat rotifers.

Discussion

Growth and Maturation

Because a male least killifish is capable of inseminating many females, and because the female can store sperm from a single copulation for many months, a female is more important for the propagation of the species than a male. Thus, a premium should be placed on the rapid growth and maturation of the females. This was found to be true. Females grew faster than males and they reached sexual maturity in 35 days, 10 days earlier than the males.

Figure 14. Monthly surface temperatures of Lake Anderson Cue at noon time.

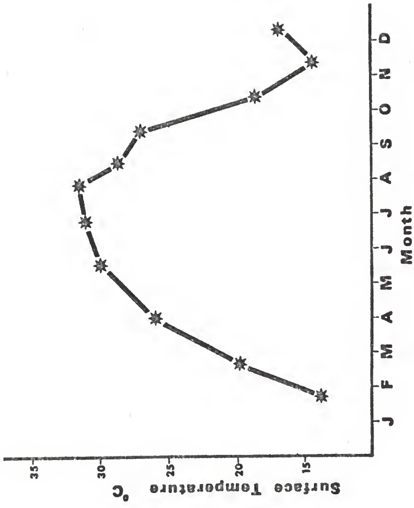
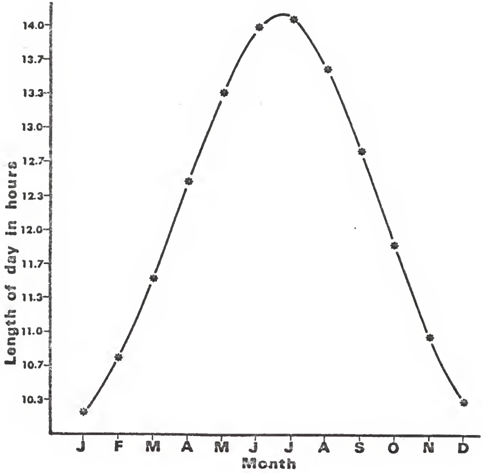

Surface Temperature °C
35
30
25
20
15
J
F
M
A
M
J
J
A
S
O
N
D
Month

Figure 15. Daylengths in hours for the first day of each month for Jacksonville, Florida.


14.0
13.7
13.3
13.0
12.7
12.3
12.0
11.7
11.3
11.0
10.7
10.3
Length of day in hours
J
F
M
A
M
J
J
A
S
O
N
D
Month

In the least killifish I found a linear relationship between the length of the female and the number of embryos she produces. It is significant that females do not cease growth after attaining sexual maturity as claimed by Brown (1957), but continue to grow throughout their life. Growth is fast during the summer when temperature and food are optimal. During the winter when temperatures are low the fish are sluggish and eat little food, even though it may be abundant. The fish grow slowly in the winter.

According to Krumholtz (1948) poeciliids, unlike most other fishes, show a definite period of "post-reproductive senility." This was not observed in the least killifish. It is possible that heavy predation in the lakes prevented the least killifish from reaching "old age." With the exception of laboratory specimens none lived more than one year. In essence, their life span beyond maturation covers their period of reproductive activity. In nature the populations do not seem to maintain post-reproductive stock, Such individuals would no longer contribute toward survival of the population but would simply compete with reproductively important individuals for food and cover.

Sex Ratios

My study like those of Geiser (1924), Hildebrand (1927), and Krumholtz (1948) on Gambusia points to differential death rates as the cause for seasonal changes in sex ratio. Neither their studies nor mine show any evidence that the fish depart from a 1:1 sex ratio at birth.

My study indicates that a high death rate for males during the winter unbalances the sex ratio and that a high death rate for females during the spring rebalances it. Males die during the winter because they are susceptible to low temperatures (p.72). One might speculate that this would enhance the survival of females during the winter by reducing competition for food and cover. However, a large proportion of the females die just prior to giving birth in the spring, which cancels any advantage that they might have gained.

It should be noted that males which spend the winter in an immature state have a longer life expectancy than females which overwinter as matures. Hoar (1957) suggests that the attainment of an early sexual maturity may be associated with a shorter life span.

Sperm Production

The proportion of the testes occupied by mature spermatozoa was lowest during May and June. I believe that sperm was used in copulation faster than it was replaced by spermatogenesis. It could also be argued that the males chosen from the two samples had not reached sexual maturity. This is unlikely as they had fully developed gonopodia. In any case the least killifish produced a quantity of sperm sufficient to inseminate females during all the months of the year.

These findings contrast with Geiser's (1924) observations made on Gambusia affinis from Beaufort, North Carolina. He found that the testes of those fish showed "marked seasonal periodicity" in both volume and spermatogensis. These differences might be species-specific or related to the more pronounced seasonal changes in day-length and temperature at his field site.

Production of Embryos

My study revealed that the least killifish has a temperature-dependent gestation period of 25 to 35 days. Fraser and Renton (1940) estimated the gestation period to be about 35 days. Gestation periods for other poeciliids are 23 to 27 days for *Lebistes reticulatus* (Rosenthal, 1951), 21 to 23 days for *Platypoecilus maculatus* (Hoover, 1943; Tavolga and Rugh, 1947), and 21 to 28 days for *Gambusia affinis* (Krumholtz, 1948).

Because of the short period of gestation and maturation, the least killifish produces generations in rapid succession. A female that overwinters can give rise to three generations of offspring before reproduction ceases in the fall. Since the females born after July 1 carry no fertilized eggs, though they have been inseminated, no fourth generation is produced. The rapid succession of generations helps to compensate for the short life and the small number of offspring a female produces.

Raitt (1932) stated that in oviparous teleosts, the number of eggs increases as the cube of the length of the mother. This generalization obviously does not apply to all species. Fecundity is related to the amount of care or nurture given to the eggs (Lagler, Bardach, and Miller, 1962). The spectrum ranges from pelagic ocean spawners which release millions of small eggs, with little food reserve, into the open sea, to nest building, mouthbreeding, and viviparous species which produce a small number of well supplied and protected eggs. In the latter there is a distinct departure from Raitt's relationship.

My data and those of Krumholtz (1948) on the western mosquitofish suggest that in poeciliids the relationship between the number of eggs and the length of the female is not exponential but rather linear. For their survival the poeciliids depend on low mortality of eggs and quick succession of generations rather than on large numbers of eggs and long life expectancy of adults.

If the body size of young females from a particular population were indicative of their age, my data would suggest a cyclical production of broods. The data indicate that large numbers of developing embryos cause successive broods to be smaller while small numbers of developing embryos allow successive broods to be large. This could be the basis for a pattern in which small broods alternate with large broods. It is probable that developing embryos slow down and suppress the maturation of eggs, since in the presence of large broods fewer eggs reach maturity than in the presence of small broods.

Turner (1937) postulated a similar explanation. He feels that brood production in poeciliid fishes might be controlled by two major internal factors, a "follicle stimulating" hormone and the retarding influence which embryos exert on unfertilized eggs. The hormone production could be influenced by external factors and thus would control the seasonal production of embryos. In its presence eggs and embryos would develop rapidly. Turner found that developing embryos counteracted the influence of this hormone by causing eggs to halt development at specific stages. In Gambusia, for example, he found that eggs would not grow larger than 0.3 mm in diameter until the brood in the ovary was released. Thus eggs "piled up" at certain stages of development and broods were large. He suggested that embryos exert a small retarding effect in species with superfetation.

My study revealed that females which had overwintered produced 79 per cent of the season's offspring. I believe that their high productivity was the direct result of their large body size at the time of year when the external stimuli favored embryo production most. Later, when their offspring reached maturity, external factors were less favorable for reproduction.

The study revealed that the least killifish reproduced from March 1 to mid-November in Lakes McCloud and Anderson Cue. These results agree with those obtained by McLane (1955) for the least killifish in the St. John's River system in northern Florida. He found that two other poeciliids, Gambusia affinis and Mollienesia iatipinna, have a similar reproductive season.

Mature eggs in young females were no longer fertilized after August 1. This was good evidence that the reproductive season was coming to a close. As the water temperature remained near 30°C from May through September, I assume that the rapidly decreasing daylength was the major factor that terminated reproduction. Older females yielded fertilized eggs until October 1. This indicates that there might be a lag between receiving the external stimulus and its effect. It might take some time to stop the production of hormones and cancel their effects. Additional evidence for the importance of daylength as an external factor in halting reproduction came from the population of least killifish that stopped reproducing in the homothermal Ichatucknee Springs (p. 48) at the same time as the lake populations.

McLane (1955) found that Gambusia, unlike the other poeciliids, continued to reproduce throughout the winter in homothermal springs along the St. John's River. Medlen (1951) also concluded that temperature was the major factor regulating reproduction in Gambusia. As Gambusia and Heterandria almost certainly speciated before ranging into the temperate zone, they could have evolved different mechanisms for control of their reproductive cycles.

The least killifish probably depends on both rising temperature and increasing daylength to initiate reproduction in the spring. In Ichatucknee the least killifish started reproduction shortly after daylength began to increase, two months sooner than in Lakes McCloud and Anderson Cue. Once daylength begins to increase, I believe reproduction will start as soon as the water temperature rises to a favorable level.

LABORATORY STUDIES

Introduction

Laboratory experiments were carried out to clarify some of the results of the field studies. I studied the survival of male and female least killifish exposed to adverse temperatures and the effects of daylength and temperature on their reproduction during the summer and outside the reproductive season.

The Effect of Water Temperature on the Survival of Heterandria

Materials and Methods

For a study of the survival of the least killifish under adverse water temperatures, I used 10 males and 25 females. They had been kept at 25°C for 43 days. On January 16, 1968, I placed the fish in 10-gallon tanks. The water was gradually brought to temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35°C by use of environmental chambers and thermostatically controlled aquarium heaters. At no time was the rate of temperature change more than 5°C per day. A 16-hour photoperiod was maintained. Surviving fish were counted after 20 and 40 days.

Fish that survived 40 days were considered acclimatized to the temperature of their tanks. Groups of five males and five females were removed from the 15°, 25°, and 35°C tanks along with 500cc of their water and placed into a lethal water bath of 38.8°C. The water in each of the three containers was saturated with oxygen from an air bubbler. The bath consisted of an aquarium containing four inches of water held at 38.8±0.5°C by use of a Blue M Constant Flow Cooling Unit in conjunction with two tank heaters. Airstones were used to circulate the water in the bath. The time to death was recorded for each fish and the average resistance times between males and females were compared.

Results

The females survived the period of acclimatization better than the males (Table 6). All the fish died within 20 days at 5°C. At 10° and 15°C the fish were very sluggish and spent most of the time lying motionless on the bottom; they ate no food. Forty per cent of the males and 36 per cent of the females died during the first week at 35°C. Although the remaining fish of the 35°C test were very thin, they survived till the end of the experiment.

For the males from the 15°C tank I found a significantly higher mean resistance time than for the females ($P=0.05$); Table 7. The females kept at 35°C had a higher resistance than the males from the same tank ($P=0.01$). At 25°C I found no significant difference.

The fish from the 15°C and 35°C tanks behaved differently when placed in the lethal bath. The former swam violently about their container and breathed rapidly. Just before death they lost their equilibrium, floated belly up to the surface, and died. The fish from the 35°C tank appeared normal for many hours; they even fed. For several hours prior to death these fish lay quietly at the surface,

Table 6

Survival of 10 male and 25 female least killifish after 20 and 40 days of exposure to various acclimatization temperatures.

Water Temperature	Per cent surviving after 20 days		Per cent surviving after 40 days	
	Males	Females	Males	Females
5°C	0.0	0.0	0.0	0.0
10°	40.0	96.0	40.0	88.0
15°	80.0	84.0	70.0	84.0
20°	50.0	88.0	30.0	80.0
25°	90.0	88.0	90.0	84.0
30°	80.0	100.0	80.0	100.0
35°	60.0	64.0	60.0	64.0

Table 7

Periods of resistance of male and female Heterandria formosa acclimatized to temperatures of 15°, 25°, and 35°C and exposed to a lethal temperature of 38.8°C.

Females

Order of Death	Resistance Time in Minutes at 38.8°C 15°C	25°C	35°C
1	37	380	1095
2	38	380	1125
3	40	560	1245
4	45	560	1268
5	47	650	1300
6			1350
Total	207	2530	7383
Mean	41.8	494	1225

Males

Order of Death	Resistance Time in Minutes at 38.8°C 15°C	25°C	35°C
1	41	260	950
2	43	470	980
3	45	510	980
4	57	530	1010
5	60	550	1040
6			1130
Total	246	2320	6090
Mean	49.2	464	1015

their fin and opercular movements gradually diminishing. At the moment of death, determined by the cessation of the opercular beat, they were still in an upright position.

Discussion

According to Brett (1941), the upper lethal limit is the highest temperature at which just 50 per cent of a population survive indefinitely. Of the least killifish kept at 35°C, 40 per cent died during the first week and the rest survived for 40 days; I believe that 35°C is close to the upper lethal limit. Upper lethal limits for other fish as given by Doudoroff (1957) are as follows: Carassius auratus, 41°C; Ictalurus nebulosus, 37.5°C; Gambusia affinis, 37.3°C; cyprinids of the genera Pimephales and Hyborhynchus, 33.2° to 33.3°C; Girella nigricans, 31.4°C; cyprinids of the genera Rhinichthys, Semotilus, and Notropis, 29.3° to 31°C; Salvelinus fontinalis, 25.3°C; and Oncorhynchus, 23.8° to 25.1°C. The least killifish has a relatively high upper lethal limit. This is of significance for a subtropical fish living in shallow water. The lower lethal limit lies between 5° and 10°C. In nature the least killifish is rarely exposed to water temperatures below 10°C, but temperatures above 35°C are fairly common.

As in Gibson's (1954) experiments with Lebistes reticulatus, I found a difference in mean resistance time of the two sexes of Heterandria formosa. Gibson's guppy males withstood low lethal temperatures better than females, but they succumbed more quickly to high lethal temperatures. I found that least killifish females withstood both low and high lethal temperatures better than males. I also found that the females were more resistant to extended periods of thermal stress than males, but were less resistant to thermal shock.

The least killifish would rarely if ever encounter thermal shock in natural habitats within its present range. Even in the winter when the surface film may be 15° to 20°C warmer than the underlying water, it is unlikely that a fish would be trapped in this layer long enough to die from shock.

The ability to withstand long-term thermal stress may be important. Since the least killifish lives in shallow water, it may be exposed to temperatures as high as 40°C for several hours each day. Should the fish try to avoid the high temperature by moving to deeper water they would be more vulnerable for predators. I once observed least killifish swimming in a shallow Everglades pool where the temperature rose to 105°F for several hours every day. As the pool had been isolated for several weeks and there was no escape to deeper water or to shade, I assumed that at least part of the population could survive exposure to such high temperatures. In such situations survival of females is particularly important to the species. The experimental results showing females more resistant to adverse temperatures than males, can be considered of ecological significance. These results might also explain the high ratios of females to males during the winter months.

Reproduction of Heterandria formosa

1. Effects of Daylength and Temperature During the Summer

Materials and methods

On June 20, 1968, a large collection of least killifish was taken from Lake McCloud and brought into the laboratory. The fish were held under conditions of natural daylength and temperature for 10 days. Then 10 males and 25 females were placed in each of six 10-gallon aquaria. Three of the tanks were set up in each of two environmental rooms maintained at $15 \pm 1^{\circ}C$. In each room one aquarium was left at $15^{\circ}C$; the water temperatures in the others were raised to 22.5° and $30^{\circ}C$ by use of thermostatically controlled aquarium heaters. One room was illuminated for eight hours and the other for 16 hours each day with two 40-watt fluorescent lamps laid across the tops of the aquaria. The light intensity at the bottom of each tank was 900 lux.

On September 8, 1968, after 70 days in the experiment, the fish were fixed in 10 per cent formalin. The total length of each fish was measured to the nearest millimeter. The ovaries were dissected and the embryos classified and counted. The testes were sectioned and stained with Heidenhain's iron hematoxylin.

Field collections were made on July 1 and September 16 for comparisons with the laboratory results.

Results

The field collection from July 1 indicated that embryos of all sizes must have been present at the beginning of the experiment and that nearly all mature females in Lake McCloud were reproducing (Tables 8 and 9). Females in the July 1 collection contained more younger than mature embryos because a large number of the young females were just starting production. Their embryos were all of the earliest stages of development.

Females kept under long days at 30°C contained large numbers of embryos in all stages of development, while those under short days had only a few embryos all of which were in the most mature age class.

Females held under long days at 22.5°C had a smaller number of embryos of all age classes while those under short days contained no class 1 embryos and only a few class 2 embryos.

Some of the females at 15°C under both long and short photoperiods contained embryos of all classes. But the number of embryos per female and the per cent of females containing embryos were low. Many of their embryos were hard and calcareous, or they were milky in appearance, and had been dead for some time. No young were born at 15°C.

The field collection from September 16 revealed a decrease in embryo production compared to that of the July 1 collection. The absence of embryos in young females indicated that the end of the reproductive season was near.

Table 8

Average number of embryos of various age classes from females kept under photoperiods and temperatures in various combinations.

Sample	Temperature °C	Average total length (mm)	Average number of embryos per female in each age class 1	2	3	4	Average number of embryos per female
July 1 field collection	31.0°C	18.3 mm	1.64	1.27	1.30	0.97	5.18
Laboratory 16-hour day	15.0°	19.9	0.31	0.31	0.18	0.44	1.25
	22.5°	20.3	2.19	2.25	1.81	2.57	8.82
	30.0°	22.5	4.69	4.44	3.69	4.37	17.19
Laboratory 8-hour day	15.0°	20.1	0.29	0.26	0.32	0.27	1.15
	22.5°	21.0	0.00	0.29	1.57	2.14	4.00
	30.0°	22.4	0.00	0.00	0.00	0.38	0.38
September 16 field collection	27.5°	17.8	0.58	0.64	0.69	0.89	2.81

Table 9

Percentage of females with embryos of various age classes kept under several combinations of photoperiod and temperature.

Sample	Temperature $^{\circ}C$	Percentage of females containing embryos in age category 1	2	3	4	Percentage of females containing embryos
July 1 field collection	$31.0^{\circ}C$	75.8	66.7	60.7	42.4	100.0
Laboratory 16-hour day	15.0°	31.2	31.2	18.7	43.7	56.3
	22.5°	75.0	75.0	68.8	93.8	100.0
	30.0°	100.0	93.7	93.7	93.7	100.0
Laboratory 8-hour day	15.0°	28.2	32.1	27.1	34.6	55.1
	22.5°	0.0	21.4	57.2	60.7	78.6
	30.0°	0.0	0.0	0.0	12.5	12.5
September 16 field collection	27.0°	37.3	44.8	46.3	52.3	74.6

Judged from the condition of the testes, all of the males were still able to reproduce at the end of the experiment.

Discussion

The fish did not reproduce at 15°C regardless of the length of the photoperiod. Temperature set a limit below which reproduction could not take place.

The absence of class 1 embryos at 22.5° and 30°C under short-day conditions indicated that fertilization did not occur when the females were under an 8-hour photoperiod. The presence of class 2 and 3 embryos in females kept under an 8-hour photoperiod at 22.5°C, but not in those at 30°C, showed that embryonic development proceeded faster at higher temperatures than at low temperatures. Under the long photoperiod of 16 hours fertilization will take place if the temperature is favorable.

Both low temperature and short photoperiods stopped reproduction. The temperatures were still high on September 16 when reproduction in the field populations declined. Therefore, it is probable that photoperiod plays a more important role than temperature in halting reproduction in the fall.

In most of the teleosts studied the eggs required a full year to develop. The single, short spawning period is terminated when all of the eggs have been released. In these species internal factors, more than daylength or temperature, bring the reproductive period to a close; for example, *Phoxinus laevis* (Bullough, 1939, 1940), *Notropis bifrenatus*

(Harrington, 1950, 1957), and Enneacanthus obesus (Harrington, 1956). It appears that gamete depletion commonly terminates the reproductive season among the more northerly species of the temperate zone.

Many subtropical fishes complete several to many short ovogenetic cycles in a year. External stimuli can be important in both starting and stopping reproduction. The greenthroat darter ceased reproduction both in the field and in the laboratory when the water temperature rose above 24°C, but it was not affected by photoperiod (Hubbs and Strawn, 1957). Turner (1957) found that Jenynsia lineata held at a constant temperature of 70°F would cease reproduction after two months when the photoperiods were shortened.

2. Effects of Daylength and Temperature During the Winter

Materials and methods

On December 4, 1966, several hundred least killifish were captured in Lake McCloud and brought into the laboratory where they were kept at 25°C under a 16-hour photoperiod. On January 16, 1967, embryos in early stages of development were taken from half of the 25 females examined. On the same day groups of 10 males and 25 females were placed in each of seven 10-gallon aquaria. Four of the tanks were placed in environmental chambers and the water was gradually cooled to temperatures of 5°, 10°, 15°, and 20°C at a rate of 5°C per day. The other tanks were placed in a room held at 25°C by air conditioning. One tank was left at 25°C and the others were raised to 30° and 35°C by use of aquarium heaters. For 40 days the fish were kept under a 16-hour photoperiod within 1.5°C of the desired temperature. Each tank was equiped with a subgravel filter, an aerator, and a dense cover of Myriophyllum. The fish were fed Wardley's aqua pellets once a day.

On February 25 the experiment was terminated and the fish were fixed in formalin. The total length of each fish was measured to the nearest millimeter, and embryos were classified and counted.

Results

The females from the field sample taken on December 4 contained no embryos, although sperm was present in the folds of the oviduct wall. The water temperature in Lake McCloud was 16°C.

Embryo development was initiated after a 43-day exposure to a 16-hour photoperiod and a water temperature of 30°C. Fifty per cent of the females became gravid and contained small broods of one to three embryos in early stages of development.

Mortality was low in the second phase of the experiment except at 5°C where all of the fish died within two weeks, and at 35°C where 40 per cent of the fish died.

In females exposed to temperatures of 10° and 15°C for 40 days, the embryos died and turned milky white. Embryonic development was initiated and continued in the fish kept at 20°, 25°, and 30°C. At the end of the experiment 34 offspring were in the 25°C tank and 39 in the 30°C tank. Three were observed after 20 days in the 20°C tank but had been lost by the end of the experiment. The females in the 30°C tank had the highest average number of embryos (Table 10). With decreasing temperature the production of embryos dropped rapidly. The 30°C tank also contained the largest number of gravid females (Table 10), but brood sizes were small and intervals between broods were long.

Discussion

High temperature and long photoperiod reactivated reproduction in least killifish that had recently ceased reproducing under natural conditions. However, the productivity of these females was quite low. The fish kept at 30°C under a 16-hour day contained an average of 3.5 embryos per female compared to 17.19 embryos per female for fish taken during June and exposed to similar conditions.

In many species of temperate zone fishes there is a short period following spawning during which the fish is physiologically incapable of reproduction. This refractory period has been observed in Phoxinus laevis (Bullough, 1939), Notropis bifrenatus (Harrington, 1957), and Enneacanthus obesus (Harrington, 1956).

Medlen (1951) found that the mosquito-fish would not reproduce in the autumn at College Station, Texas, although the temperature was sufficiently high. He theorized that the females might have "spent" themselves and needed some time for gonadal recuperation.

I believe that the reduced productivity of least killifish taken in the winter might be caused in a similar manner. The ovaries

Table 10

Productivity of winter females under a 16-hour photoperiod at various water temperatures.

Water Temperature	Average Number of Embryos per Female	Percentage of Females Containing Embryos
5°C	(all died)	(all died)
10°	0.682	22.7
15°	1.19	47.6
20°	1.26	47.4
25°	1.91	61.9
30°	3.24	84.0
35°	0.00	0.0

contained very few eggs at the end of the reproductive season. Therefore, only a few could be fertilized when the fish were exposed to conditions favorable for reproduction.

The lower temperature range at which reproduction stopped was between 15° and 20°C, while the upper limit was between 30° and 35°C. Medlin (1951) found the lower limit for reproduction in the mosquitofish to be near 16°C. I found the optimum temperature for the production of embryos to be near 30°C. The temperatures of the lakes in the field study remained near 30°C during most of the reproductive season.

GENERAL DISCUSSION

The results of the field and laboratory studies showed that seasonal changes in productivity of the least killifish were caused by the influence of daylength and temperature upon the female. Sperm production did not vary significantly. A lack of seasonal variation in spermatogensis has been found in the Indian catfish (Ghosh and Kar, 1952) and in Fundulus confluentus (Harrington, 1959a). Harrington (1959a) suggested that lack of seasonal change in the reproductive condition of males may be widespread among "low-latitude" fishes. In fishes living at "high-latitudes" seasonal changes in the testes are usually detectable, but they are not as pronounced as in the ovaries.

In a species such as the least killifish in which the females are capable of storing sperm for long periods of time the effects of daylength and temperature on sperm production would not be critical for regulating reproduction. Any factor exerting seasonal control on reproduction would have to act on the female either by regulating the stage to which eggs are able to mature or by activating and deactivating stored sperm.

The female least killifish has an inherent rhythm of ovogenesis which is reinforced by seasonal changes in daylength and temperature. Increasing daylength and high temperatures stimulate reproduction while decreasing daylength and low temperatures inhibit it. When one of the two external factors receeds below the threshold for stimulation, fertilization will not take place. In Lakes McCloud and Anderson Cue temperature was the last to rise above the threshold in the spring while daylength was the first to drop below the threshold in the fall. In the homothermal spring of Ichatucknee, the temperature remained above the threshold level. Therefore, changes in daylength must be responsible for starting and ending reproduction.

The reproductive cycle of the least killifish is most similar to that of Jenynsia lineata, another live-bearer, In both species reproduction can be initiated or terminated by increasing or decreasing photoperiods if the water temperature is kept constantly high. Although Turner (1957) did not investigate effects of low temperature on Jenynsia, it seems reasonable that it would inhibit embryo production.

In reviewing the literature, I found that a control of reproductive cycles in teleosts of the north temperate zone is very rigid. Most species must be exposed to a sequence of different daylengths and temperatures for their eggs to pass through all stages of development. During the early stages of the ovogenetic cycle any exposure of the females to optimal stimuli for reproduction will not make the eggs to mature (Harrington, 1956, 1957, 1959b; Baggerman, 1957). The majority of the species depend on changing daylength or on a combination of changing daylength and temperature to initiate their short spawning periods (Hoover and Hubbard, 1937; Hazard and Eddy, 1951; Bullough, 1939; Eeckhoudt, 1946; Baggerman, 1957;

Harrington, 1950, 1956, 1957, 1959b). Very few species depend predominately on temperature (Ahsan, 1966a; Merriman and Schedl, 1941). Daylength is a more regular variable than temperature and permits a more precise control.

Among "subtropical" species, temperature appears to be the most commonly used external factor for regulating reproductive cycles (Medlen, 1951; Hubbs and Strawn, 1957; Harrington, 1959a). Here mistakes in the timing of reproduction are less disastrous and many species have a long reproductive season (McLane, 1955; Medlen, 1951; Harrington, 1959a). Control tends to be less rigid.

For a "subtropical" fish, the least killifish has evolved a surprisingly precise method of controlling reproduction.

SUMMARY

Field Studies

1. Monthly field collections of least killifish were taken from Lakes McCloud and Anderson Cue near Melrose, Florida. The fish were measured, sexed, and checked for embryos. Thin sections were made of the male's testes.

2. Females matured in 35 days when they were on the average 14 mm long; males matured in 45 days and averaged 15 mm in total length. Growth was fastest during April and slowest from December to March. The females continued to grow until death; the males ended their growth shortly after they reached sexual maturity. None of the fish examined was more than one year old and none survived the reproductive phase.

3. Sex ratios fluctuated seasonally; females were predominant during the winter. Since the sex ratio was approximately 1:1 at birth, a differential death rate was responsible for the changing sex ratio.

4. There was no pronounced seasonal change in the condition of the testes; they were always in their active state.

5. Embyros were produced from the beginning of March to mid-November; production peaked in May and June. The gestation period varied from 25 to 35 days. The average number of embryos per female was directly proportional to their total length. Females which had overwintered produced 79 per cent of the seasons offspring. Productivity dropped off steadily from June to November as old females were replaced by their offspring.

6. Least killifish taken from the homothermal Ichatucknee Spring terminated reproduction in October, but resumed it again by January 1.

7. It was concluded that decreasing daylength terminated reproduction in the fall and that increasing daylength and high temperatures initiated it in the spring.

Laboratory Studies

Effect of Water Temperature on the Survival of Heterandria

1. Ten males and 25 females were acclimatized to temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35°C for 40 days. Survival was checked at 20 and 40 days. Fish from the 15°, 25°, and 35°C tanks were exposed to a lethal temperature of 38.8°C.

2. The upper and lower lethal limits were about 35°C and 5° to 10°C respectively. Females survived acclimatization better than males. Males resisted thermal shocks better than females; the latter were more resistant to long-term thermal stress.

Reproduction of Heterandria formosa

Effects of daylength and temperature during the summer

1. Ten males and 25 females were kept at temperatures of 15°, 22.5°, and 30°C under 16-hour and 8-hour photoperiods for 70 days.

2. The condition of the testes remained unchanged under all combinations of photoperiod and temperature.

3. Embryos were produced only at 22.5°C and 30°C under a 16-hour photoperiod. It was concluded that low temperatures and short days terminated reproduction.

Effects of daylength and temperature during the winter

1. On December 4 a large collection of least killifish were exposed to a temperature of 25°C and a 16-hour photoperiod. They were then held for 40 days at temperatures ranging from 5°C to 35°C.

2. Exposure to water at 25°C and a 16-hour photoperiod caused a low rate of embryo production. The fish seemed to remain in a partially refractory period. They reproduced when held at 20°, 25°, and 30°C, and reached the peak at 30°C.

Both high temperatures and increasing photoperiod were necessary to maintain embryo production in the female least killifish. In contrast, these external factors had very little effect on sperm production.

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BIOGRAPHICAL SKETCH

Carl M. Colson was born May 11, 1943, at Falls City, Nebraska. In June, 1961, he was graduated from Humboldt High School. In June, 1965, he received the degree of Bachelor of Science with a major in Zoology from the University of Nebraska. In September, 1965, he enrolled in the Graduate School of the University of Florida. From September, 1965, until the present time he has pursued his work toward the degree of Doctor of Philosophy as a National Science Foundation Graduate Fellow.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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